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EFFECT OF CIRCUMSTANCES AND PROCESS ON THE CONTENT OF CHEMICAL COMPONENTS IN BAI SHAO (*PAEONIA LACTIFLORA* PALL.)

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Abstract

Effects of circumstances and process on the content of chemical components in Bai Shao (*Paeonia lactiflora* Pall.) collected from different locations of China were studied. The amount of gallic acid, catechin, albiflorin, paeoniflorin, benzoic acid and paeonol were processed and determined with RP-HPLC. The result showed that there were differences between the content of chemical components in Bai Shao in different locations. Meteorological condtions and age of Bai Shao affected the content of chemical components. The content of albiflorin in Bai Shao increases along with the decrease of rainfall, integral air temperature and the increase of age. The climate, age and process affect the quality of Bai Shao. The process facilitates the realization of medicinal property and function of Bai Shao.

Introduction

Bai Shao is the processed root of cultivated *Paeonia lactiflora* Pall. belongs to Paeoniaceae (Chinese Pharmacopoeia Committee 2015). The processing of *P. lactiflora* root involves scratching the epidermis after boiling at 90 - 100ºC for 10 - 12 min. The main chemical components of Bai Shao are benzoic acid, gallic acid, ethyl gallate, paeoniflorin, oxypaeoniflorin, benzoyl paeoniflorin, albiflorin, 1, 2, 3, 4, 6-pentagalloyglucose, etc. (Wen-xiang *et al*. 2000, Qiao *et al*. 2007). Bai Shao can recuperate liver and relieve pain, nurish blood and recuperate meridian system, astringe, hemostasis sweat. Besides, it can also be used to cure headache, dizziness, hypochondriac, bellyache, limb spasm pain, blood deficiency, chlorosis, irregular menses, night sweat and spontaneous perspiration (Chinese Pharmacopoeia Committee 2015). The plant yielding Bai Shao is cultivated in Bozhou, Hangzhou, Dongyang, Zhongjiang, Shanxi province, Ganshu province, Jilin province and so on in China. The cultivation area of Bai Shao in Bozhou occupy over 80% of that in China.

P. lactiflora and *Paeonia lactiflora* Pall. var. *trichocarpa* (Bunge) F. C. Stern are cultivated in most of the parts of Bai Shao in China. Farmers do not differentiate *P. lactiflora* and *P. lactiflora* var. *trichocarpa*. The contents of paeoniflorin in Bai Shao in different locations are diverse (Yue-feng *et al*. 2005, Yi-feng *et al*. 2007). However, there are many medicinal ingredients such as gallic acid and albiflorin in Bai Shao in addition to paeoniflorin. Their contents are related to geographical locations. Furthermore, the processing affects the efficacy of Bai Shao (Gui-yang *et al*. 2005). Therefore, there is difference between the contents of chemically active ingredients in Bai Shao and that of raw root of *P. lactiflora*. The present investigation has therefore been undertaken to investigate Bai Shao collected from different geographical locations of China to find the effects of growing locations and processing on chemically active ingredients. The main goal of the present work was to establish factors affecting the Bai Shao for a harmonious quality control.

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Materials and Methods

The raw roots of *P. lactiflora* and *Paeonia lactiflora* var. *trichocarpa* from different locations were collected from September to October in 2009. These locations were not privately-owned or protected and the species collected were not endangered or protected. Ten plants were collected randomly from each location. Roots of those plants in each location were dried (50ºC, 48 hrs in drying oven), crushed and mixed together with each other as composite sample representive to each location. The Bai Shao materials of all locations were bought from traders (it needs time, equipment and technique to process the roots of *P. lactiflora*). The process must be done by producer. The species of these materials were identified by Prof. Wang Wenquan, a researcher in Beijing University of Chinese Medicine.

Medicinal material for control (came from *P. lactiflora*) (batch number 121093 - 200402) was purchased from National Institute for the Control of Pharmaceutical and Biological Products in China in March 2009. This institution is thought as authority on standard.

The conditions of HPLC: High performance liquid chromatograph (Agilent 1100, Agilent Technologies), HPLC column (Diamonsil C18, 5 μm, 250 mm × 4.6 mm, cat. no.: 99903, Ser. no.: 813184, Dikma Technologies Company), mobile phrase consisted of acetonitrile (HPLC grade) and 0.2% phosphoric acid (v/v). The content of acetonitrile in gradient mobile phrase varied as below: from 5 to12% in 0 - 18 min; 12 to 15% in 18 - 22 min; 15% in 22 - 32 min; from 15 to 20% in 32 - 45 min; 20% in 45 - 60 min; from 20 to 40% in 60 - 80 min; 40% in 80 - 82 min. The flow rate was 1 ml/min with detection wavelength set at 230 nm. The temperature of HPLC column was same as room temperature. Each sample injected was 5 μl. The plate was higher than 5000 when paeoniflorin was determined.

Methods of establishing standard curve: 1.65 mg/l paeoniflorin solution, 0.205 mg/l albiflorin solution, 0.1 mg/l catechin solution, 0.85 mg/l gallic acid, 0.865 mg/l benzoic acid solution and 0.350 mg/l benzoic acid solution were prepared. Then each of the solutions was diluted to 3/4, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128 times. All of the standard solutions were determined as the method 1.3.3 and the peak area of each standard was integrated. The standard curve of each standard was established by SPSS.

Methods of extraction and determination of chemical components in Bai Shao: The materials were crushed and passed through a 80 meshes sieve after being dried at 50ºC. 0.2 g material was immersed in 20 ml of 50% ethanol for 30 min and refluxed for 2 hrs at 90ºC. The extract was filtered with filter paper, and then the filtrate was evaporated with a rotary evaporator (RE-52A, Shanghai Yarong Biochemistry Instrument Factory) under low barometric pressure (75ºC, 0.08 MPa). Each sample was extracted twice. The resultant was dissolved with 10 ml methanol and filtered using a 0.45 μm membrane filter. These extracts were determined with the above HPLC method.

Precision of HPLC: The solution of reference compounds was precisely prepared. The concentration of paeoniflorin, albiflorin, catechin, gallic acid and benzoic acid solutions was set at 0.20, 0.10, 0.10, 2.00 and 0.30 mg/l, respectively in reference compound solution. Then these reference compounds were determined using the method 2.3.2 six times. The value of peak areas corresponding to 5 components in each spectrum were recorded and the RSD (relative standard deviation) of the areas of each component was analyzed by SPSS (statistic software). The RSD of paeoniflorin, albiflorin, catechin, gallic acid and benzoic acid solutions was 2.16, 2.44, 3.79, 2.54 and 2.61%, respectively.

Results and Discussion

Thirteen materials were collected and investigated. The locations of these materials occupy the most locations of all Bai Shao yielding species in China. The information of the Bai Shao material is shown in Table 1.

Table 1. Geomorphological and climatological variables of the locations of Bai Shao yielding species in China.

Species	Location	Terrain	Elev.	Lat. and Long.	Soil	Rainfall	Air temp.	Integral temp.
P. lactifera	Heze	Flatland	50	N35°17′1″, E115°31′37″	Alluvial soil	650	14	4560
P. lactiflora	Bozhou	Flatland	50	N33°47′57″, E115°49′20″	Alluvial soil	780	14.7	4704
P. lactiflora *P. trichocarpa*	Dongyang	Hillside	410	N29°2′57″, E120°20′3″	Red soil	1480	16.1	5640
P. lactiflora *P. trichocarpa*	Zhongjiang	Hillside	1030	N31°0′50″, E104°33′30″	Yellow soil	940	16.3	5280
P. lactiflora	Hanzhong	Flatland	810	N32°59′30″, E107°20′40″	Yellow brown soil	960	14.2	4440
P. lactiflora *P. trichocarpa*	Dunhua	Flatland	490	N43°30′45″, E128°8′40″	Planosol	620	3.5	2448
P. lactiflora	Tianshui	Hill	1250	N34°38′35″, E105°53′12″	Loessial soil	560	10.7	3696
P. trichocarpa	Wuniute	Flatland	650	N42°25′17″, E118°43′15″	Yellow loessail soil	380	7.2	3072
P. lactiflora *P. trichocarpa*	Luoyang	Flatland	130	N34°41′3″, E112°20′24″	Alluvial soil	620	14.7	4680
P. lactiflora	Anguo	Flatland	50	N38°21′31″, E115°18′3″	Alluvial soil	490	12.4	4440
P. lactiflora	Longtoushan	Hillside	1130	N41°57′34″, E117°40′23″	Eluvial cinnamon soil	420	5.8	2688
P. lactiflora	Yudaokou	Grassland	1484	N42°21′28″, E117°07′50″	Dark chestnut soil	460	1.4	1944
P. lactiflora	Beijing	Flatland	90	N39°59′25″, E116°12′28″	Yellow loessail soil	620	12	4320

The conditions of HPLC and the extraction method were verified to determine the contents of chemical components in Bai Shao. The chromatogram of standards is shown in Fig. 1. The representative chromatogram of Bai Shao is shown in Fig. 2.

The contents of gallic acid, catechin, albiflorin, paeoniflorin, benzoic acid and paeonol in Bai Shao and these in raw materials in all locations were determined with HPLC. The results are presented in Table 2.

There are significant difference between the contents of gallic acid (or benzoic acid) in materials in Heze, Bozhou, Dongyang or Zhongjiang.

There are significant difference among the main chemical components in raw Bai Shao materials according to the result of HPLC (Fig. 3). The content of paeoniflorin in Bozhou is higher than that in Hangzhou and Zhongjiang. Most of all these paeoniflorin contents in all locations are over the standard (1.6%) stipulated in Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee 2015). The contents of catechin in Bai Shao materials in all locations are very low, even lower than detection limit.

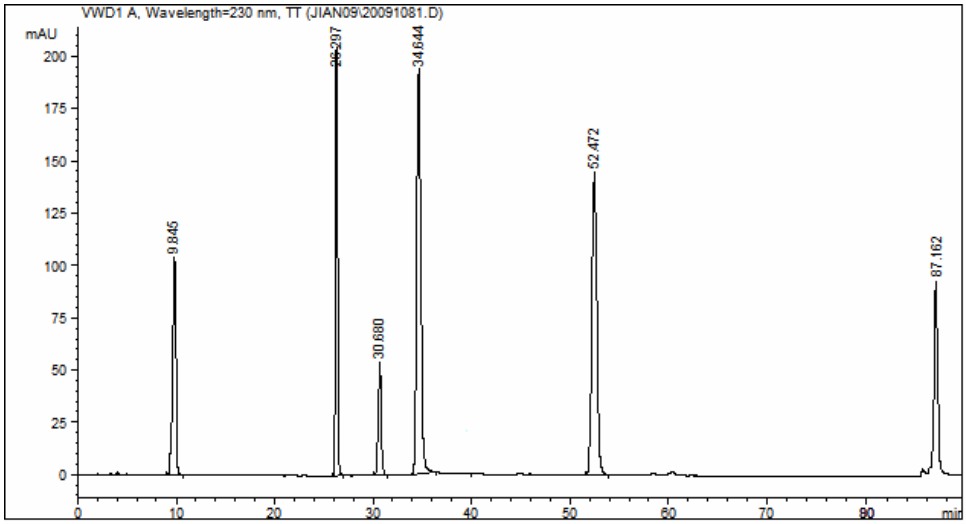

Fig. 1. Chromatogram of standards (From left to right: gallic acid, catechin, albiflorin, paeoniflorin, benzoic acid and paeonol).

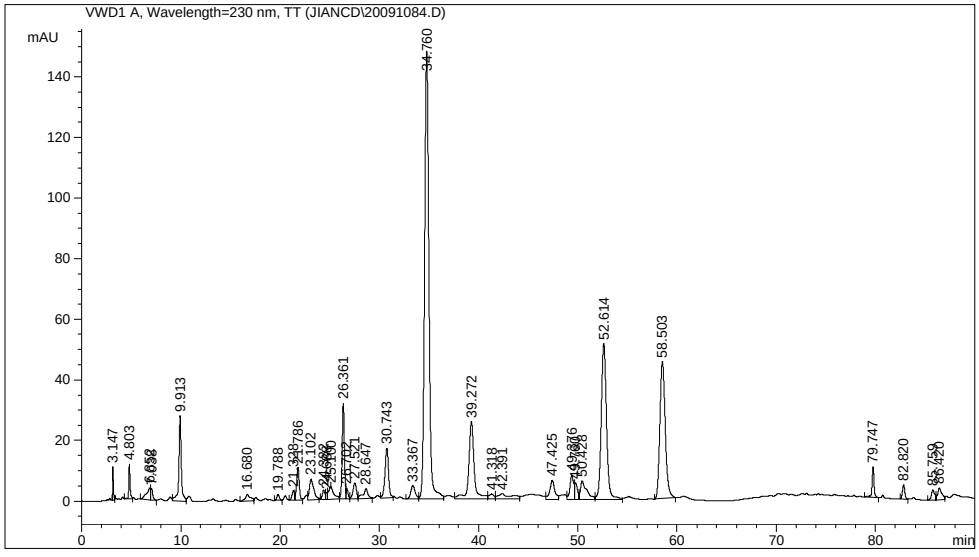

Fig. 2. Chromatogram of Bai Shao.

The contents of gallic acid, catechin, albiflorin, paeoniflorin, benzoic acid and paeonol in Bai Shao and these in raw materials in all locations were determined with HPLC. The results are presented in Table 2.

Table 2. Difference between the contents of main components in Bai Shao and raw materials from different locations.

Location	Species, age	Gallic acid	Catechin	Albiflorin	Paeoni-florin	Benzoic acid	Paeonol
Heze	Raw *lactiflora*, 5	0.1202	0.0024	0.7773	2.8816	0.0788	0.0186
	Raw *lactiflora*, 6	0.1277	0.0151	1.7543	4.9967	0.0441	0.0200
	Raw *lactiflora*, 6	0.0730	0.0563	1.5426	3.8529	0.0999	0.0171
Bozhou	Raw *lactiflora*, 4	0.1088	-	0.7155	2.4987	0.0793	0.0221
	Raw *lactiflora*, 4	0.2107	-	0.8694	3.7734	0.1074	—
	Raw *lactiflora*, 4	0.2058	-	0.9387	3.7889	0.1026	0.0088
	Processed *lactiflora*, 4	0.0749	-	0.3342	2.5709	—	0.0183
Dongyang	Raw *lactiflora*, 4	0.1423	-	0.3024	2.0201	0.0286	0.0128
	Processed *lactiflora*, 4	0.0687	0.0293	0.7110	2.7326	0.0099	0.0313
Zhongjiang	Raw *lactiflora*, 4	0.2077	-	1.3765	2.3302	0.0830	0.0219
	Raw *Trichocarpa*, 4	0.2105	-	0.9588	3.3468	0.0785	0.0159
	Processed *lactiflora*,4	0.1663	-	1.0685	2.5455	-	0.0252
Hanzhong	Raw *lactiflora*, 3	0.3406	-	1.1639	1.5787	0.0617	0.0098
	Processed *lactiflora*, 4	0.2596	-	0.7307	2.9786	0.0122	0.0270
Dunhua	Raw *lactiflora*, 5	0.1032	-	0.4349	2.2052	0.0439	0.0143
Tianshui	Raw *lactiflora*, 5	0.1514	0.0454	1.0359	3.3540	0.0413	0.0307
Wongniute	Raw *Trichocarpa*, 3	0.1480	0.0276	0.9828	3.3246	0.0413	0.0136
Luoyang	Raw *Trichocarpa*, 5	0.1590	0.0390	0.4733	4.3760	0.0743	0.0089
Anguo	Raw *lactiflora*, 4	0.1974	0.0172	1.3721	3.0941	0.1401	0.0116
Longtoushan	Raw *lactiflora*, 2	0.2192	0.0101	1.7311	2.8728	0.0901	-
Yudaokou	Raw *lactiflora*, 2 (root crop)	0.2400	0.0073	1.6093	3.1342	0.1787	0.0259
	Raw *lactiflora*, 2 (seedling)	0.1506	0.0333	2.9875	2.2590	0.4001	0.0235
Beijing	Raw *lactiflora*, 4	0.1320	0.0288	0.6051	1.8299	0.0303	0.0140

"-"Shows that the content was not determined. *Shows significant at the 0.05 level (2-tailed).

There is significant positive correlation between the content of paeoniflorin in raw Bai Shao and age according to the results of correlation between circumstances and the contents of main components in raw Bai Shao (Table 3). Simultaneously, there is significant negative correlation between the content of gallic acid (or benzoic acid) and Bai Shao age. The correlation between the content of catechin in raw Bai Shao and age is not obvious. There is significant negative correlation between the contents of albiflorin (or benzoic acid) and the average temperature of air (or integral temperature). The significant negative correlation between the content of paeoniflorin in Bai Shao and rainfall appeared also.

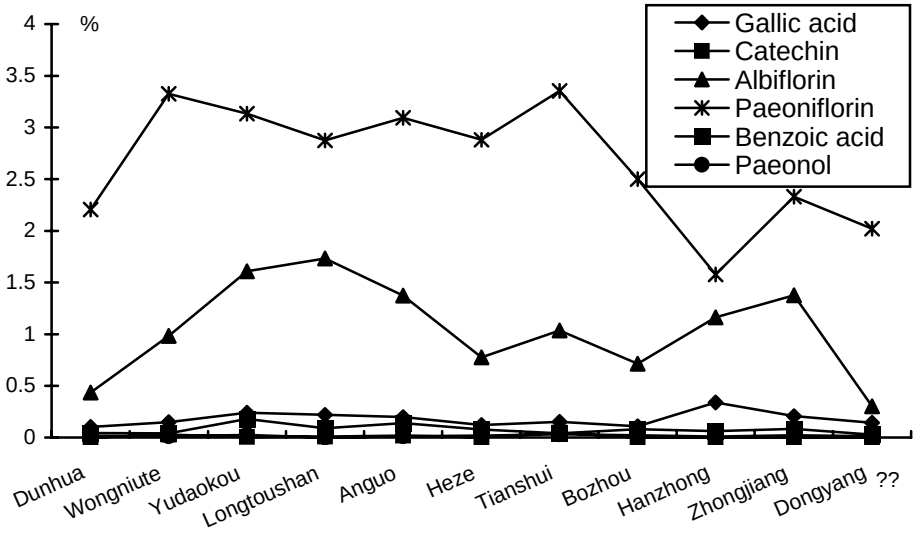

Fig. 3. The contents of main constituents in raw Bai Shao in different locations.

Table 3. The correlation between circumstance and the contents of main components constituents in raw Bai Shao.

Factors	Gallic acid	Catechin	Albiflorin	Paeoniflorin	Benzoic acid	Paeonol
Latitude	–0.141	–0.338	0.363	–0.096	0.357	0.128
Longitude	–0.453	–0.462	–0.101	–0.083	0.072	–0.211
Planting age	–0.588**	0.407	–0.415	0.457*	–0.524*	–0.034
Elevation	0.409	–0.059	0.515*	–0.307	0.435	0.571*
Rainfall	0.138	0.242	–0.444	–0.29	–0.336	–0.265
Average temperature of air	0.019	0.236	–0.482*	0.195	–0.525*	–0.356
Integral temperature	–0.011	0.214	–0.501*	0.134	–0.508*	–0.372
Gallic acid	1	–0.556	0.147	–0.21	0.092	–0.195
Catechin	–0.556	1	–0.036	0.152	–0.005	–0.009
Albiflorin	0.147	–0.036	1	0.084	0.792**	0.44
Paeoniflorin	–0.21	0.152	0.084	1	–0.086	0.018
Benzoic acid	0.092	–0.005	0.792**	–0.086	1	0.292
Paeonol	–0.195	–0.009	0.44	0.018	0.292	1

*Correlation is significant at 0.05 level (2-tailed); **Correlation is significant at 0.01 level (2-tailed).

The contents of main chemical components in Bai Shao are affected by many factors such as environment, planting age and so on. This reveals that environment affects the quality of Bai Shao. The results from the experiment are consistent with some reports (Kangcai *et al*. 2008). Wang Kangcai considered that benzoic acid was distributed in the epidermis of *P. lactiflora* root. The content of benzoic acid reduced after boiling and scratching the epidermis. Therefore, the contents of benzoic acid in processed Bai Shao are lower than that in raw Bai Shao material (Kangcai *et al*. 2008). Meng Xiangsong considered that *P. lactiflora* root should be dried after washing to reduce the loss of paeoniflorin (Xiang-song and Lei 2008). Zhao Yanan also considered that scratching the

epidermis of Bai Shao should be studied (Ya-nan and Jian 2001). Benzoic acid is poisonous or harmful in Chi Shao (dry root of *P. lactiflora*) or food to person (Zhen-hua *et al*. 2008, Na 2006). The processing reduces the content of benzoic acid, increases the content of paeoniflorin, even changes the chemical components in Bai Shao. The processing often contribute to the performance and function of Bai Shao different from to Chi Shao. Therefore, it was considered that processing is necessary to manufacture Bai Shao and the processing should be specified.

The results also showed that there is positive correlation between the contents of albiflorin and benzoic acid in raw Bai Shao material (Table 3). There is positive correlation between the contents of paeoniflorin and catechin in Bai Shao.

The contents of gallic acid and benzoic acid in processed Bai Shao are significantly lower than that of raw Bai Shao material (Fig. 4). There is no obvious difference between the paeoniflorin contents in processed and raw Bai Shao materials.

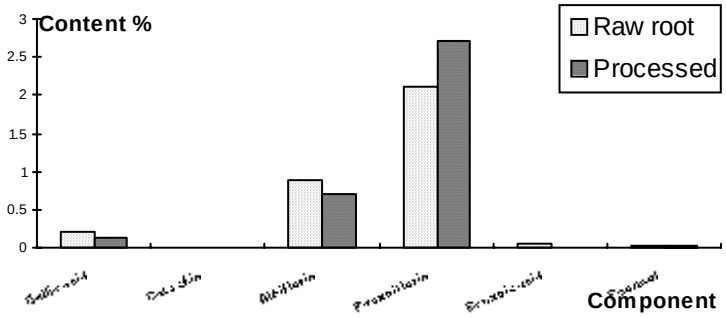

Fig. 4. The difference between the contents of main chemical component constituents in raw and processed Bai Shao.

The results showed that there is positive correlation between the contents of paeoniflorin and catechin Bai Shao (Table 3) and it is consistent with that in Chi shao reported earlier (Jian *et al*. 2013). There is also positive correlation between the contents of albiflorin and benzoic acid in raw Bai Shao material. These phenomenon reveal that there is relationship between some chemical components in *P. lactiflora*. The relationship can be used in establishing quality evaluation system of Bai Shao.

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